

- 35. (Added) A method for producing a human-like glycoprotein in a lower eukaryotic host cell that does not display a 1,6 mannosyltransferase activity with respect to the N-glycan or a glycoprotein, the method comprising the step of introducing into the host cell one or more enzymes for production of a Man₅GlcNAc₂ carbohydrate structure, wherein at least 30% Man₅GlcNAc₂ is produced within the host cell which can serve as a substrate for GnT1 in vivo.
- 36. (Added) The method of claim 35, wherein at least one of the enzymes is selected to have optimal activity at the pH of the location in the host cell where the carbohydrate structure is produced.
- 37. (Added) The method of claim 36, wherein at least one of the enzymes is selected to have a pH optimum within 1.4 pH units of the average pH optimum of other representative enzymes in the organelle in which the enzyme is localized.
- 38. (Added) The method of claim 35, wherein the enzyme is targeted by means of a cellular targeting signal peptide not normally associated with the enzyme.
- 39. (Added) The method of claim 35, wherein at least one introduced enzyme is targeted to the endoplasmic reticulum, the early, medial, late Golgi or the trans Golgi network of the host cell.
- 40. (Added) The method of claim 39, wherein at least one of the enzymes is selected from the group consisting of mannosidases, glycosyltransferases and glycosidases.

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- 41. (Added) The method of claim 39, wherein the enzyme is a mannosidase predominantly localized in the Golgi apparatus or the endoplasmic reticulum.
- 42. (Added) The method of claim 35, wherein the glycoprotein comprises N-glycans having fewer than six mannose residues.
- 43. (Added) The method of claim 35, wherein the glycoprotein comprises N-glycans having fewer than four mannose residues.
- 44. (Added) The method of claim 35, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, sialic acid, and fucose.
- 45. (Added) The method of claim 35, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.
- 46. (Added) The method of claim 35, wherein the host is selected from the group consisting of Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Candida albicans, Aspergillus nidulans, and Trichoderma reesei.
- 47. (Added) The method of claim 35, wherein the host is deficient in the activity of one or more enzymes selected from the group consisting of mannosyltransferases and phosphomannosyltransferases.

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- 48. (Added) The method of claim 47, wherein the host does not express an enzyme selected from the group consisting of 1,6 mannosyltransferase; 1,3 mannosyltransferase; and 1,2 mannosyltransferase.
- 49. (Added) The method of claim 35, wherein the host is an OCH1 mutant of P. pastoris.
- 50. (Added) The method of claim 35, wherein the host expresses GnTI and a UDP-GlcNac transporter.
- 51. (Added) The method of claim 35, wherein the host expresses a UDP-or GDP-specific diphosphatase.
- 52. (Added) The method of claim 35, further comprising the step of isolating the glycoprotein from the host.
- 53. (Added) The method of claim 52, further comprising the step of subjecting the isolated glycoprotein to at least one further glycosylation reaction *in vitro*, subsequent to its isolation from the host.
- 54. (Added) The method of claim 35, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more enzymes for production of the Man₃GlcNAc₂ carbohydrate structure.
- 55. (Added) The method of claim 35, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more mannosidase

enzymes involved in the production of Man₅GlcNAc₂ from Man₈GlcNAc₂ or Man₉GlcNAc₂.

- 56. (Added) The method of claim 55, wherein at least one of the encoded mannosidase enzymes has a pH optimum within 1.4 pH units of the average pH optimum of other representative enzymes in the organelle in which the mannosidase enzyme is localized, or has optimal activity at a pH between 5.1 and 8.0.
- 57. (Added) The method of claim 56, wherein the mannosidase enzyme has optimal activity at a pH between 5.9 and 7.5.
- 58. (Added) The method of claim 56, wherein the mannosidase enzyme is an α -1,2-mannosidase derived from mouse, human, Lepidoptera, Aspergillus nidulans, or Bacillus sp.
- 59. (Added) The method of claim 54, wherein at least one enzyme is localized by forming a fusion protein between a catalytic domain of the enzyme and a cellular targeting signal peptide.
- 60. (Added) The method of claim 59, wherein the fusion protein is encoded by at least one genetic construct formed by the in-frame ligation of a DNA fragment encoding a cellular targeting signal peptide with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.
- 61. (Added) The method of claim 54, wherein the catalytic domain encodes a glycosidase or glycosyltransferase that is derived from a member of the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, Fucosyltransferase

and ST, and wherein the catalytic domain has a pH optimum within 1.4 pH units of the average pH optimum of other representative enzymes in the organelle in which the enzyme is localized, or has optimal activity at a pH between 5.1 and 8.0.

- 62. (Added) The method of claim 54, wherein the nucleic acid molecule encodes one or more enzymes selected from the group consisting of UDP-GlcNAc transferase, UDP-galactosyltransferase, GDP-fucosyltransferase, CMP-sialyltransferase, UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, CMP-sialic acid transporter, and nucleotide diphosphatases.
- 63. (Added) The method of claim 54, wherein the host expresses GnTI and a UDP-GlcNac transporter.
- 64. (Added) The method of claim 54, wherein the host expresses a UDP-or GDP-specific diphosphatase.

REMARKS

In the October 2, 2002 Office Action, the Examiner required restriction of original claims 1-34 into one of the following five inventions, pursuant to 35 U.S.C. § 121:

Group I: Claims 1-23, drawn to a method of producing a glycoprotein having carbohydrate structures similar to human cells by introducing one or more enzymes for production of said carbohydrate structure into a fungal host lacking said enzymes;